

INTERNATIONAL SEARCH REPORT

International Application No

T/GB 99/02785

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/543 G01N27/327 C12Q1/28 C12Q1/58 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GUISEMPI-ELIE, A. ET AL: "Electroconductive polymer thin films with internal bioactive moieties for biosensor applications" POLYM. MATER. SCI. ENG. (1995), 72, 404-5 , XP000853822 the whole document	1,8,9, 19,20, 24,25,41
A	WO 98 35232 A (NOVALON PHARMACEUTICAL CORP ;UNIV NORTH CAROLINA (US); FOWLKE DAN) 13 August 1998 (1998-08-13) examples	1,8,9, 19,20, 24,25,41
A	US 5 312 762 A (GUISEMPI-ELIE ANTHONY) 17 May 1994 (1994-05-17) abstract	1,8,9, 19,20, 24,25,41

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the International search

Date of mailing of the International search report

25 November 1999

03/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5018 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax (+31-70) 340-3016

Authorized officer

Moreno, C

INTERNATIONAL SEARCH REPORT

International Application No

PT/GB 99/02785

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 89 11649 A (WOLLONGONG UNIADVICE) 30 November 1989 (1989-11-30) cited in the application the whole document	1,8,9, 19,20, 24,25,41
A	EP 0 193 154 A (CHEMO SERO THERAPEUT RES INST) 3 September 1986 (1986-09-03) cited in the application the whole document	1,8,9, 19,20, 24,25,41

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/02785

Patent document cited in search report		Publication dat		Patent family member(s)		Publication dat
WO 9835232	A	13-08-1998		AU 6651798 A NO 993764 A		26-08-1998 28-09-1999
US 5312762	A	17-05-1994		WO 9306237 A CA 2048692 A WO 9010655 A US 5766934 A US 5352574 A		01-04-1993 14-09-1990 20-09-1990 16-06-1998 04-10-1994
WO 8911649	A	30-11-1989		NONE		
EP 0193154	A	03-09-1986		JP 1961890 C JP 6097219 B JP 61195346 A AT 72333 T CA 1241057 A DE 3683656 A		25-08-1995 30-11-1994 29-08-1986 15-02-1992 23-08-1988 12-03-1992

TENT COOPERATION TRE

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year)
18 April 2000 (18.04.00)

To:
Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

International application No.
PCT/GB99/02785

Applicant's or agent's file reference
SCB/51312001

International filing date (day/month/year)
24 August 1999 (24.08.99)

Priority date (day/month/year)
24 August 1998 (24.08.98)

Applicant

FARMAKOVSKI, Dmitri Alexandrovich et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

22 March 2000 (22.03.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

S. Mafla

Telephone No.: (41-22) 338.83.38

TENT COOPERATION TRE

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

BOULT WADE TENNANT
 Verulam Gardens
 70 Gray's Inn Road
 London, WC1X 8BT
 ROYAUME-UNI

Date of mailing (day/month/year)
 03 May 2000 (03.05.00)

Applicant's or agent's file reference
 SCB/51312001

International application No.
 PCT/GB99/02785

IMPORTANT NOTIFICATION

International filing date (day/month/year)
 24 August 1999 (24.08.99)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address

BOULT WADE TENNANT
 27 Furnival Street
 London EC4A 1PQ
 United Kingdom

State of Nationality State of Residence

Telephone No.
 0171-430 7500

Faxsimile No.
 0171-831 1768

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address

BOULT WADE TENNANT
 Verulam Gardens
 70 Gray's Inn Road
 London, WC1X 8BT
 United Kingdom

State of Nationality State of Residence

Telephone No.
 0171-430 7500

Faxsimile No.
 0171-831 1768

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

 the receiving Office the International Searching Authority the International Preliminary Examining Authority the designated Offices concerned the elected Offices concerned other:

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Kari Huynh-Khuong

Telephone No.: (41-22) 338.83.38

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION	
International application No. PCT/GB99/02785	International filing date (day/month/year) 24 August 1999
(Earliest) Priority date (day/month/year) 24 August 1998	
Title of invention METHOD OF ELECTROCHEMICAL ANALYSIS OF AN ANALYTE	
Box No. II APPLICANT(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) SENSOR-TECH LIMITED PO Box 301, Don Road St Helier Jersey JE4 8UG Channel Islands	Telephone No.: Facsimile No.: Teleprinter No.:
State (that is, country) of nationality: Jersey - CHANNEL ISLANDS	State (that is, country) of residence: JERSEY - CHANNEL ISLANDS
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) FARMAKOVSKI; DMITRI ALEXANDROVICH Flat 46, Building 48 Kastanaevskaya Street 1121108 Moscow Russian Federation	
State (that is, country) of nationality: RUSSIA	State (that is, country) of residence: RUSSIA
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) MILANOVSKI; YEVGENI YUREVICH Flat 46, Building 48 Kastanaevskaya Street 1121108 Moscow Russian Federation	
State (that is, country) of nationality: RUSSIA	State (that is, country) of residence: RUSSIA
<input type="checkbox"/> Further applicants are indicated on a continuation sheet.	

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

CHERKASOV; VLADIMIR RURIKOVICH
 Flat 46, Building 48
 Kastanaevskaya Street
 1121108 Moscow
 Russian Federation

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
RUSSIA

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

BIRYUKOV; YURI SERGEYEVICH
 Flat 46, Building 48
 Kastanaevskaya Street
 1121108 Moscow
 Russian Federation

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
RUSSIA

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

LEONARDOVA; OLGA
 401, 1830-11 AVE.SW
 Calgary
 Alberta T3C 0N6
 CANADA

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
CANADA

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

BALDOCK; SHARON CLAIRE
 27 Furnival Street
 London EC4A 1PQ
 United Kingdom

State (that is, country) of nationality:
UK

State (that is, country) of residence:
UK

Further applicants are indicated on another continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is agent common representative

and has been appointed earlier and represents the applicant(s) also for international preliminary examination.

is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BOULT WADE TENNANT
VERULAM GARDENS
70 GRAY'S INN ROAD
LONDON WC1X 8BT
UNITED KINGDOM

Telephone No.:

+44 (0)20 7430 7500

Facsimile No.:

+44 (0)20 7831 1768

Teleprinter No.:

Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION**Statement concerning amendments.***

1. The applicant wishes the international preliminary examination to start on the basis of:

the international application as originally filed

the description as originally filed
 as amended under Article 34

the claims as originally filed
 as amended under Article 19 (together with any accompanying statement)
 as amended under Article 34

the drawings as originally filed
 as amended under Article 34

2. The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

- * Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

- which is the language in which the international application was filed.
 which is the language of a translation furnished for the purposes of international search.
 which is the language of publication of the international application.
 which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|--------------------------------------------------------------------------|---|----------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | 1 sheets |
| 6. other (specify) | : | sheets |

For International Preliminary Examining Authority use only

received	not received
<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below:

- | | |
|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and or amino acid sequence listing in computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 6. <input checked="" type="checkbox"/> other (specify): Debit Order No. 14799/P |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

BALDOCK; SHARON CLAIRE
Authorised Representative

For International Preliminary Examining Authority use only

- | | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|--|
| 1. Date of actual receipt of DEMAND: | | |
| 2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b): | | |
| 3. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. | <input type="checkbox"/> The applicant has been informed accordingly. | |
| 4. <input type="checkbox"/> The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5. | | |
| 5. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82. | | |

For International Bureau use only

Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No.	PCT/GB99/02785	For International Preliminary Examining Authority use only
Applicant's or agent's file reference	SCB/NLW/51312/001	Date stamp of the IPEA
Applicant SENSOR-TECH LIMITED		
Calculation of prescribed fees		
1. Preliminary examination fee	1533 Euros	P
2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>)	148 Euros	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	1681 Euros	TOTAL
Mode of Payment		
<input type="checkbox"/> authorization to charge deposit account with the IPEA (see below)	<input type="checkbox"/> cash	
<input type="checkbox"/> cheque	<input type="checkbox"/> revenue stamps	
<input type="checkbox"/> postal money order	<input type="checkbox"/> coupons	
<input type="checkbox"/> bank draft	<input checked="" type="checkbox"/> other (<i>specify</i>): Debit Order No. 14799/P	

Deposit Account Authorization (*this mode of payment may not be available at all IPEAs*)

The IPEA/ is hereby authorized to charge the total fees indicated above to my deposit account.

(*this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit*) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

Deposit Account Number	Date (day/month/year)	Signature
------------------------	-----------------------	-----------

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) SCB/51312001

Box No. I TITLE OF INVENTION

METHOD OF ELECTROCHEMICAL ANALYSIS OF AN ANALYTE

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

SENSOR-TECH LIMITED
P.O. BOX 301
DON ROAD
ST. HELIER
JERSEY JE4 8UG
CHANNEL ISLANDS

This person is also inventor.

Telephone No.

Faximile No.

Teleprinter No.

State (that is, country) of nationality:

JERSEY-CHANNEL IS.

State (that is, country) of residence:

JERSEY-CHANNEL IS.

This person is applicant
for the purposes of:

all designated
States

all designated States except
the United States of America

the United States
of America only

the States indicated in
the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

FARMAKOVSKI; DMITRI ALEXANDROVICH
FLAT 46, BUILDING 48
KASTANAEVSKAYA STREET
1121108 MOSCOW
RUSSIAN FEDERATION

This person is:

applicant only

applicant and inventor

inventor only (If this check-box
is marked, do not fill in below.)

State (that is, country) of nationality:

RUSSIA

State (that is, country) of residence:

RUSSIA

This person is applicant
for the purposes of:

all designated
States

all designated States except
the United States of America

the United States
of America only

the States indicated in
the Supplemental Box

Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf
of the applicant(s) before the competent International Authorities as:

agent

common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation.
The address must include postal code and name of country.)

BOULT WADE TENNANT
27 FURNIVAL STREET
LONDON EC4A 1PQ
UNITED KINGDOM

Telephone No.

0171-430 7500

Faximile No.

0171-831 1768

Teleprinter No.

267271 Boult G

Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the
space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

MILANOVSKI; YEVGENI YUREVICH
FLAT 46, BUILDING 48,
KASTANAEVSKAYA STREET
1121108 MOSCOW
RUSSIAN FEDERATION

This person is:

- applicant only
 applicant and inventor

inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
RUSSIA

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

CHERKASOV; VLADIMIR RURIKOVICH
FLAT 46, BUILDING 48,
KASTANAEVSKAYA STREET
1121108 MOSCOW
RUSSIAN FEDERATION

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
RUSSIA

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BIRYUKOV; YURI SERGEYEVICH
FLAT 46, BUILDING 48,
KASTANAEVSKAYA STREET
1121108 MOSCOW
RUSSIAN FEDERATION

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
RUSSIA

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

LEONARDOVA; OLGA
401, 1830-11 AVE. SW
CALGARY
ALBERTA
T3C 0N6
CANADA

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
RUSSIA

State (that is, country) of residence:
CANADA

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BALDOCK; SHARON CLAIRE
27 FURNIVAL STREET
LONDON EC4A 1PQ
UNITED KINGDOM

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
GBState (that is, country) of residence:
GB

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked).

Regional Patent

- AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line).

- | | |
|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> LR Liberia |
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> LS Lesotho |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> LT Lithuania |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> LU Luxembourg |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> LV Latvia |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> MW Malawi |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> MX Mexico |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CU Cuba | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> CZ Czech Republic | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GD Grenada | <input checked="" type="checkbox"/> SL Sierra Leone |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> GH Ghana | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> GM Gambia | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> HR Croatia | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> ID Indonesia | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> IL Israel | <input checked="" type="checkbox"/> US United States of America |
| <input checked="" type="checkbox"/> IN India | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> IS Iceland | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> JP Japan | <input checked="" type="checkbox"/> YU Yugoslavia |
| <input checked="" type="checkbox"/> KE Kenya | <input checked="" type="checkbox"/> ZA South Africa |
| <input checked="" type="checkbox"/> KG Kyrgyzstan | <input checked="" type="checkbox"/> ZW Zimbabwe |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | |
| <input checked="" type="checkbox"/> KR Republic of Korea | Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet: |
| <input checked="" type="checkbox"/> KZ Kazakhstan | <input checked="" type="checkbox"/> CR Costa Rica |
| <input checked="" type="checkbox"/> LC Saint Lucia | <input checked="" type="checkbox"/> MM Myanmar |
| <input checked="" type="checkbox"/> LK Sri Lanka | |

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Supplemental Box If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ... " [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the Inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

CONTINUATION OF BOX NO. III FURTHER APPLICANTS

BALDOCK; SHARON CLAIRE
is applicant for the purposes of
LIBERIA (LR)

Box No. VI PRIORITY CLAIM		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.		
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1) 24 AUGUST 1998	98116346	RU		
item (2)				
item (3)				

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s):

* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):	Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority).		
	Date (day/month/year)	Number	Country (or regional Office)
ISA / EP			

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains
the following number of sheets:

request : 6
description (excluding sequence listing part) : 88
claims : 13
abstract : 1
drawings : 4
sequence listing part of description : _____

Total number of sheets : 112

This international application is accompanied by the item(s) marked below:

1. fee calculation sheet
2. separate signed power of attorney
3. copy of general power of attorney: reference number, if any:
4. statement explaining lack of signature
5. priority document(s) identified in Box No. VI as item(s):
6. translation of international application into (language):
7. separate indications concerning deposited microorganism or other biological material
8. nucleotide and/or amino acid sequence listing in computer readable form
9. other (specify): _____

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request):

BOULT WADE TENNANT

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: <input type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference SCB/51312001

Applicant
SENSOR-TECH LIMITED

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE

55

T

2. SEARCH FEE

638

S

International search to be carried out by EP

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 112 sheets.

first 30 sheets 285

b1

82 x 6 = 492

b2

remaining sheets additional amount

Add amounts entered at b1 and b2 and enter total at B 777

B

Designation Fees

The international application contains ALL designations.

10 x 65 = 650

D

number of designation fees amount of designation fee payable (maximum 10)

Add amounts entered at B and D and enter total at I 1427

I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is or all applicants are so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT (if applicable)

P

5. TOTAL FEES PAYABLE

2120

TOTAL

The designation fees are not paid at this time.

MODE OF PAYMENT

authorization to charge deposit account (see below)

bank draft

coupons

cheque

cash

other (specify):

postal money order

revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION (this mode of payment may not be available at all receiving Offices)

The RO/ is hereby authorized to charge the total fees indicated above to my deposit account.

(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account No.

Date (day/month/year)

Signature

PATENT COOPERATION TREATY

Miss Balogch
as 70/100
NATS 24/2/01

NB. 13/11

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

To:	RECEIVED 09 NOV 2000 BOULT WADE TENNANT
BOULT WADE TENNANT VERULAM GARDENS 70 Gray's Inn Road London WC1X8BT GRANDE BRETAGNE	

Date of mailing
(day/month/year) 06.11.2000

Applicant's or agent's file reference SCB/NLW/51312/001	IMPORTANT NOTIFICATION	
International application No. PCT/GB99/02785	International filing date (day/month/year) 24/08/1999	Priority date (day/month/year) 24/08/1998.
Applicant SENSOR-TECH LIMITED et al.		

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Digiusto, M Tel. +49 89 2399-8162	
---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------	---------------------------------------------------------------------------------------

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SCB/NLW/51312/001	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/02785	International filing date (day/month/year) 24/08/1999	Priority date (day/month/year) 24/08/1998
International Patent Classification (IPC) or national classification and IPC G01N33/543		
Applicant SENSOR-TECH LIMITED et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 13 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input checked="" type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		

Date of submission of the demand 22/03/2000	Date of completion of this report 06.11.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Tilkorn, A-C Telephone No. +49 89 2399 8688



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/02785

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-10,12-20,	as originally filed	
22-88		
11,21	with telefax of	09/10/2000

Claims, No.:

1-38	with telefax of	09/10/2000
------	-----------------	------------

Drawings, sheets:

1/4-4/4	as originally filed
---------	---------------------

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/02785

V. Reasons needed statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-9,11-38
	No:	Claims -
Inventive step (IS)	Yes:	Claims 1-9,11-38
	No:	Claims -
Industrial applicability (IA)	Yes:	Claims 1-9,11-38
	No:	Claims -

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the International application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/02785

Section I:

Claim 10 goes beyond the disclosure of the application as originally filed and therefore contravenes Art 34(2)(b) PCT:

In the original disclosure, the use of label-peroxidase has only been described in Examples 4-6 and 9. However, these examples do not provide a sufficient basis for a claim, which covers a much broader scope than the said examples.

Consequently, no opinion on novelty, inventive step and industrial applicability is given for **claim 10** in its entirety (Rule 70.2(c) PCT).

In addition, the amendment of the description (p 21 l 30) infringes Art 34(2)(b) PCT, because a technical feature (namely "horseradish peroxidase") is added to the embodiment (p 21 l 23-p 11 l 2) concerned. Thus, examination has been carried out on the basis of the description including original page 21 (Rule 70.2.(c) PCT).

Section V:

The following documents are referred to in this communication:

1 Novelty (Art 33(2)PCT):

1.1 **Claim 1** is novel, because none of the available documents discloses a method which involves a sensing electrode having an electroconductive polymer coating and which relies on monitoring the electrode potential relative to a reference electrode before and after an ion-step (steps (d) and (e)) at constant pH. For the same reason **claims 2-9, 11-38** are novel, too.

2 Inventive Step (Art 33(3)PCT):

2.1 Claim 1 appears to be inventive for the following reasons:

D1, which is considered to represent the closest prior art, pertains to the

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/02785

potentiometric detection of a non-charged analyte, namely an antigen. The apparatus used comprises an ISFET carrying an antibody-loaded membrane (abstract) the antibody corresponding to a receptor according to present claim 1. The assay is carried out in a competition format and the electrochemical detection consists of an ion-step method.

The method of **claim 1** is distinguished from the method of D1 in the sensing electrode used, as the ISFET (D1) is coated by an antibody loaded membrane whereas the electrode according to claim 1 is coated by an electroconductive polymer carrying receptors.

Moreover, the potentiometric assay of D1 is based on detecting a shift of isoelectric point of the ISFET membrane whereas according to the present invention a change of electrode potential at constant pH is determined.

As the method of D1 is performed at continuously changing pH over a pH range, it requires a large number of potentiometric measurements to be taken before and after the immune reaction (D1: p 369 col 1 "The ion-step Procedure", Fig. 3).

The problem to be solved can be regarded as the provision of a electrochemical detection method which only requires two measurements.

D2 describes electrodes being coated with electroconductive polymers, but the electrochemical detection assay of D2 is based on amperometric measurements which require a different electrochemical cell. D2 does not contain any indicator for a method involving the determination of a potential difference nor does it point to an ion-step procedure.

Thus, the solution of present **claim 1**, namely the use of an electrode coated with an electroconductive polymer and the determination of the difference in electrode potential in the presence of the analyte, is neither disclosed nor rendered obvious by any piece of prior art.

In conclusion, **claim 1** appears to satisfy Art 33(3) PCT.

For the same reasons also **claims 2-6, 37 and 38** appear to meet the requirements of Art 33(3) PCT.

- 2.2 **Claim 7** seems to comply with Art 33(3)PCT: The subject-matter of claim 7 makes use of enzyme-labelled secondary receptors, the enzyme converting a substrate to a product, which affects the redox composition of the

electroconductive polymer. Instead of a change in ionic strength of the electrolyte (claims 1 and 2), the method of claim 7 relies on the change of the redox composition of the electroconductive polymer, which covers the sensing electrode, caused by the enzyme reaction.

D2, which is considered to represent the closest prior art, describes a method that involves competing molecules being conjugated with an enzyme, e.g. horseradish peroxidase (D2: p 46 l 29-p 48 l 4). The immobilized enzyme lowers the electrochemical barrier in the redox chemistry of the substrate in the vicinity of the electrode and the electrode detects the electric potential difference (p 47 l 4-8). So, when the surrogate ligand, which corresponds to the competing molecule, binds to the immobilized receptor on the electrode, the bound enzyme is brought in sufficient proximity to electrode surface. The enzyme may then be reduced by electron transfer from the polymer of the electrode surface. Further, the electrons are transferred to the substrate, namely the hydrogen peroxide in the solution and oxygen and water are produced (p 47 l 22-28).

The method of D2 is distinguished from the subject-matter of claim 25 in the electrochemical measurement: according to D2 an external potential has to be applied that causes a current between electrode and solution which is monitored throughout the assay, whereas according to claim 25 the electric potential difference between sensing electrode and reference electrode is monitored. The method according to D2 requires electrochemical mediators in or on the electroconductive polymer coating which are capable to transfer redox equivalents from the electrode to the enzyme in order to maintain it in a reduced state in which it is catalytically active (D2: p 47 l 4-8, l 16-p 48 l 4).

The problem to be solved can thus be regarded as how to provide a method which overcomes the need for redox mediators immobilized in or on the electroconductive polymer coating of the sensing electrode.

None of the available documents renders the solution of claim 7, namely the measurement of the potential difference between sensing electrode and reference electrode. Thus, **claims 7** appears to satisfy Art 33(3) PCT. The same arguments apply to **claim 8**.

- 2.3 As **claims 9, 11-36** are all dependent on either of the independent claims 1,2, 7 or 8, which seem to comply with Art 33(3) PCT, said claims also seem to be inventive.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/02785

Section VI:

This Preliminary Report is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (24.8.1999). If it later turns out that this is not correct, the document D3 (publication date: 27.8.1998) could become relevant to assess whether the subject-matter claimed satisfies the criteria set forth in Article 33(1)PCT.

Moreover, in some cases D3 may become relevant in the regional phase (e.g. before the EPO (Art 54 (3) EPC)) for the assessment of novelty.

Section VII:

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 is not mentioned in the description, nor is this document identified therein. Moreover, the method disclosed in D1 and the major differences to the present invention should have been summarized in the description.

In order for the application to be self-contained the reference to non-published patent applications should be replaced by the corresponding publication numbers (Guidelines II 4.17)(e.g. p 2 I 6: publication number: WO 98/37409).

Section VIII:

Claim 4 does not comply with Art 6 PCT, because the expression "greater than one electrostatic unit" is not clear.

Claims 7 and 8 do not satisfy Art 6 PCT, because they lack an essential feature. From the description it is understood that the methods rely on enzymic reactions that change the redox potential of the reaction mixture.

- 11 -

aniline are the preferred monomers. Deionised water is preferably used as the polar solvent.

As is well known to persons skilled in the art, electroconductive polymers are often doped at the 5 electrochemical synthesis stage in order to modify the structure and/or conduction properties of the polymer. A typical dopant anion is sulphate (SO_4^{2-}) which is incorporated during the polymerisation process, neutralising the positive charge on the 10 polymer backbone. Sulphate is not readily released by ion exchange and thus helps to maintain the structure of the polymer. In the present invention it is preferred to use dopant anions having maximum 15 capability for ion exchange with the solution surrounding the polymer in order to increase the sensitivity of the electrodes. This is accomplished by using a salt whose anions have a large ionic radius as the background electrolyte when preparing the electrochemical polymerisation solution.

20 Suitable salts whose anions have large ionic radius include sodium dodecyl sulphate and dextran sulphate. The concentration of these salts in the electrochemical polymerisation solution is varied according to the type of test within the range 0.005 25 - 0.05 M.

As reported in a number of papers [4, 5], the ease with which ion exchange takes place and the rapidity with which ion equilibrium is attained for electroconductive polymers immersed in a solution are 30 essentially dependent on the size of the dopant anion introduced at the electrodeposition stage: the larger the ionic radius of the dopant anion, the more readily ion-exchange reactions take place and the more rapidly a state of equilibrium is reached. This 35 is directly linked to the value and rate of change of

09-10-2000

PCT/GB99/02785

- 21 -

(c) contacting the sensing electrode with a solution comprising competing molecules capable of binding to said immobilized or adsorbed receptors, said competing molecules being conjugated with an

5 enzyme;

d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and

10 e) monitoring the electric potential difference between the sensing electrode and a reference electrode following exposure to an electrolyte comprising the substrate for said enzyme.

In one embodiment of the above-described methods
15 the enzyme conjugated to the secondary receptors or competing molecules is capable of converting a substrate which directly affects the redox composition of the electroconductive polymer coating of the sensing electrode into a product which has no detectable effect on the redox composition of the
20 electroconductive polymer.

In an alternative embodiment, the enzyme conjugated to the secondary receptors or competing molecules is capable of converting a substrate which
25 has no detectable effect on the redox composition of the electrochemical polymer coating of the sensing electrode to a product capable of directly or indirectly affecting the redox composition of the electroconductive polymer. An example of such an enzyme is horseradish peroxidase. One way in which the product of the enzymic reaction may indirectly affect the redox composition of the electroconductive polymer is by causing a change in the pH of the electrolyte (for this embodiment the pH of the
30 electrolyte is not buffered). An example of an
35

- 89 -

Claims:

1. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

5 (a) providing a sensing electrode having an electroconductive polymer coating, the coating having immobilised therein or adsorbed thereto receptors which are capable of binding the desired analyte to be detected in the sample;

10 (b) contacting the sensing electrode with a test solution comprising the sample so that said desired analyte binds to said immobilised or adsorbed receptors;

15 (c) contacting the sensing electrode with a solution comprising secondary receptors capable of binding to said analyte at a site spatially distinct from the site of binding to the immobilised or adsorbed receptors, said secondary receptors being conjugated with a charge label;

20 (d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and

25 (e) monitoring the electric potential difference between the sensing electrode and a reference electrode following a change in the ionic strength of the electrolyte at constant pH.

30 2. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

35 (a) providing a sensing electrode having an electroconductive polymer coating, the coating having

- 90 -

immobilised therein or adsorbed thereto receptors which are capable of binding to the desired analyte to be detected in the sample;

5 (b) contacting the sensing electrode with a
test solution comprising the sample so that said
analyte binds to said immobilised or adsorbed
receptors;

10 (c) contacting the sensing electrode with a solution comprising competing molecules capable of binding to said immobilised or adsorbed receptors, said competing molecules being conjugated with a charge label;

20 (e) monitoring the electric potential difference between the sensing electrode and a reference electrode following a change in the ionic strength of the electrolyte at constant pH.

25 3. A method as claimed in claim 1 or claim 2
wherein the charge label has the following
properties:

(i) it carries a net charge at the pH of the electrolyte of part d); and

(ii) the magnitude of this charge changes in response to a change in the ionic strength of the electrolyte at constant pH;

35 . 4. A method as claimed in claim 1 or claim 2
wherein the charge label has a net charge at the pH
of the electrolyte of greater than one electrostatic

- 91 -

unit.

5. A method as claimed in claim 3 or claim 4
wherein the charge label is ferrocene, latex
microspheres or gold.

6. A method as claimed in any one of claims 1
to 5 wherein steps (b) and (c) are performed
simultaneously by contacting the sensing electrode
10 with a test solution to which has been added
secondary receptors or competing molecules conjugated
with a charge label.

7. A method of electrochemical detection of an
15 analyte in a sample, which method comprises the steps
of:

(a) providing a sensing electrode having an
electroconductive polymer coating, the coating having
immobilized therein or adsorbed thereto receptors
20 which are capable of binding to the desired analyte
to be detected in the sample;

25 (b) contacting the sensing electrode with a
test solution comprising the sample so that the said
analyte binds to said immobilized or adsorbed
receptors;

30 (c) contacting the sensing electrode with a
solution comprising secondary receptors capable of
binding to said analyte at a site spatially distinct
from the site of binding to immobilized or adsorbed
receptors, said secondary receptors being conjugated
with an enzyme;

35 (d) monitoring the electric potential
difference between the treated sensing electrode and
a reference electrode when both are immersed in an

09-10-2000

PCT/GB99/02785

- 92 -

electrolyte; and

5 (e) monitoring the electric potential difference between the sensing electrode and a reference electrode following exposure to an electrolyte comprising the substrate for said enzyme.

8. A method of electrochemical detection of an analyte in a sample, which method comprises the steps 10 of:

(a) providing a sensing electrode having an electroconductive polymer coating, the coating having immobilized therein or adsorbed thereto receptors which are capable of binding to the desired analyte 15 to be detected in the sample;

(b) contacting the sensing electrode with a test solution comprising the sample so that the said 20 desired analyte binds to said immobilized or adsorbed receptors;

(c) contacting the sensing electrode with a solution comprising competing molecules capable of 25 binding to said immobilized or adsorbed receptors, said competing molecules being conjugated with an enzyme;

(d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an 30 electrolyte; and

(e) monitoring the electric potential difference between the sensing electrode and a reference electrode following exposure to an 35 electrolyte comprising the substrate for said enzyme.

- 93 -

9. A method as claimed in claim 7 or claim 8 wherein the enzyme is capable of converting a substrate which has no detectable effect on the redox composition of the electroconductive polymer coating of the sensing electrode to a product capable of directly or indirectly affecting the redox composition of the said electroconductive polymer coating.

10 10. A method as claimed in claim 9 wherein the enzyme is a peroxidase.

15 11. A method as claimed in claim 9 wherein the product capable of indirectly affecting the redox composition of the electroconductive polymer membrane causes a change in the pH of the electrolyte of part (e).

20 12. A method as claimed in claim 11 wherein the enzyme is a urease.

25 13. A method as claimed in claim 7 or claim 8 wherein the enzyme is capable of converting a substrate which has no detectable effect on the redox composition of the electroconductive polymer coating of the sensing electrode to a product which is a substrate for a second enzyme, the action of the second enzyme generating a second product which directly or indirectly affects the redox composition 30 of the electroconductive polymer coating of the sensing electrode.

35 14. A method as claimed in claim 7 or claim 8 wherein the enzyme is capable of converting a substrate which directly affects the redox composition of the electroconductive polymer coating of the sensing electrode to a product which has no

09-10-2000

- 94 -

detectable effect on the redox composition of the said electroconductive polymer coating.

15. A method as claimed in any one of claims 1
5 to 14 wherein the receptor molecules are monoclonal
antibodies, polyclonal antibodies, antibody
fragments, antibody mimics, chimaeric antibodies
viral lysates, recombinant proteins, synthetic
peptides, hormones, hormone receptors, single
stranded nucleic acids, low molecular weight
molecules, chemical compounds conjugated with
10 proteins (haptens), fragments of bacterial, plant or
animal cells, lectins, glycoproteins or
carbohydrates.

15

16. A method as claimed in any one of claims 1 to 15 wherein the electroconductive polymer coating of the sensing electrode has been doped with dopant anions.

20

17. A method as claimed in claim 16 wherein the dopant anions are dodecyl sulphate or dextran sulphate.

25

18. A method as claimed in any one of claims 7 to 17 wherein steps (b) and (c) are performed simultaneously by contacting the sensing electrode with a test solution to which has been added secondary receptors or competing molecules conjugated with an enzyme label.

३५

19. A method as claimed in any one of claims 1 to 18 wherein the sensing electrode comprises adaptor molecules immobilized in or absorbed to the electroconductive polymer coating thereof and the receptors capable of binding to the analyte to be detected are attached to the said adaptor molecules.

- 95 -

20. A method as claimed in claim 19 wherein steps (b) and (c) are performed simultaneously with a step of contacting the sensing electrode with receptors by contacting the sensing electrode having adaptor molecules immobilised in or adsorbed to the electroconductive polymer layer with a test solution to which has been added receptors and secondary receptors or competing molecules conjugated with a charge label or enzyme.

10

21. A method as claimed in claim 19 or claim 20 wherein the adaptor molecules are molecules capable of binding to at least one class of receptor molecules capable of binding to the said analyte.

15

22. A method as claimed in claim 19 or claim 20 wherein the receptors capable of binding to the analyte to be detected are biotinylated, the adaptor molecules are avidin or streptavidin and the receptors are attached thereto via a biotin/avidin or biotin/streptavidin binding interaction.

25

23. A method as claimed in claim 19 or claim 20 wherein the receptors capable of binding to the analyte to be detected are antibodies, the adaptor molecules are protein A or protein G and said antibodies are attached thereto via a protein A/antibody or protein G/antibody binding interaction.

30
35

24. A method as claimed in claim 19 or claim 20 wherein the receptors capable of binding to the analyte to be detected contain a sugar moiety, the adaptor molecules are lectins and the receptors are attached thereto via a lectin/sugar binding interaction.

25. A method as claimed in claim 19 or claim 20

- 96 -

wherein the receptors capable of binding to the analyte to be detected are labelled with FITC, the adaptor molecules are anti-FITC antibodies and the receptors are attached thereto via an FITC/anti-FITC binding interaction.

5

26. A method as claimed in any one of claims 1 to 25 in which biological fluids such as whole blood, serum, lymph, urine, saliva, cerebrospinal fluid or semen are used as the test solution.

10

27. A method as claimed in any one of claims 1 to 26 wherein at least steps (d) and (e) are carried out in a flow-through measuring cell.

15

28. A method as claimed in any one of claims 19 to 27 wherein the step of providing a sensing electrode having adaptor molecules immobilized in the electroconductive polymer coating comprises producing the said electrode using a method comprising steps of:

20
25

(a) preparing an electrochemical polymerisation solution comprising monomeric units of the electroconductive polymer and adaptor molecules,

30
35

(b) immersing an electrically conductive electrode in the electrochemical polymerisation solution, and

(c) applying a cyclic electric potential between the said electrode and the electrochemical polymerisation solution to coat the electrode by electrochemical synthesis of the polymer from the solution, said cyclic electric potential being applied for at least one full cycle.

- 97 -

29. A method as claimed in any one of claims 19 to 27 wherein the step of providing a sensing electrode having adaptor molecules adsorbed to the electroconductive polymer coating comprises producing the said electrode using a method comprising steps of:

(a) preparing an electrochemical polymerisation solution comprising monomeric units of the electroconductive polymer,

(b) immersing an electrically conductive electrode in the electrochemical polymerisation solution,

(c) applying a cyclic electric potential between the electrode and the electrochemical polymerisation solution to coat the electrode by electrochemical synthesis of the polymer from the solution, said cyclic electric potential being applied for at least one full cycle; and

(d) contacting the coated electrode with a solution comprising adaptor molecules such that the adaptor molecules are adsorbed onto the electroconductive polymer coating of the electrode.

30. A method as claimed in claim 28 or claim 29 wherein the adaptor molecules are selected from the group consisting of avidin, streptavidin, anti-FITC antibodies and a molecule capable of specifically binding to at least one class of receptor molecules.

35 31. A method as claimed in any one of claims 28 to 30 wherein monomeric units of the electroconductive polymer are pyrrole, thiophene, furan or aniline.

- 98 -

32. A method as claimed in any one of claims 28 to 31 in which a dopant salt is added to the electrochemical polymerisation solution.

5 33. A method as claimed in claim 32 wherein the salt is sodium dodecylsulphate or sodium dextran sulphate.

10 34. A method as claimed in any one of claims 28 to 33 wherein the cyclic electric potential has a sawtooth form.

15 35. A method as claimed in any one of claims 28 to 34 wherein the cyclic electric potential is applied for at least two cycles.

20 36. A method as claimed in any one of claims 28 to 35 wherein the cyclic electric potential has a peak value applied to the electrode which is less than or equal to +2 volts.

25 37. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

30 (a) providing a sensing electrode comprising an electrically conductive electrode coated with a layer of electroconductive polymer with molecules of avidin or streptavidin immobilized therein or adsorbed thereto, said avidin or streptavidin molecules being attached to receptor molecule capable of binding the analyte to be detected attached via a biotin/avidin or biotin/streptavidin binding interaction;

35 (b) contacting the sensing electrode with a test solution comprising the sample so that said desired analyte binds to said immobilized or adsorbed receptor molecules;

09-10-2000

PCT/GB99/02785

- 99 -

(c) monitoring the potential of the sensing electrode relative to a reference electrode when both are immersed in an electrolyte; and

5 (d) monitoring the potential difference of the sensing electrode relative to the reference electrode following a change in the ionic strength or composition of the electrolyte at constant pH.

10 38. A method as claimed in claim 37 wherein the analyte to be detected is a nucleic acid and the receptor molecules are oligonucleotides.

TOTAL P.22

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference SCB/51312001	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, Item 5 below.	
International application No. PCT/GB 99/02785	International filing date (day/month/year) 24/08/1999	(Earliest) Priority Date (day/month/year) 24/08/1998
Applicant SENSOR-TECH LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the International search was carried out on the basis of the International application in the language in which it was filed, unless otherwise indicated under this item.
 - the International search was carried out on the basis of a translation of the International application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any nucleotide and/or amino acid sequence disclosed in the International application, the International search was carried out on the basis of the sequence listing :
 - contained in the International application in written form.
 - filed together with the International application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the International application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of invention is lacking (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

8

Non of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

A sensing electrode for use in methods of electrochemical analysis comprising an electrically conducting electrode coated with an electroconductive polymer membrane having immobilised therein or adsorbed thereto adaptor molecules avindin, streptavidin, anti-fitc antibodies through which the sensing electrode can be made specific for an analyse under test by the binding of receptors specific for the analyse.

- 11 -

aniline are the preferred monomers. Deionised water is preferably used as the polar solvent.

As is well known to persons skilled in the art, electroconductive polymers are often doped at the 5 electrochemical synthesis stage in order to modify the structure and/or conduction properties of the polymer. A typical dopant anion is sulphate (SO_4^{2-}) which is incorporated during the polymerisation process, neutralising the positive charge on the 10 polymer backbone. Sulphate is not readily released by ion exchange and thus helps to maintain the structure of the polymer. In the present invention it is preferred to use dopant anions having maximum 15 capability for ion exchange with the solution surrounding the polymer in order to increase the sensitivity of the electrodes. This is accomplished by using a salt whose anions have a large ionic radius as the background electrolyte when preparing the electrochemical polymerisation solution.

20 Suitable salts whose anions have large ionic radius include sodium dodecyl sulphate and dextran sulphate. The concentration of these salts in the electrochemical polymerisation solution is varied according to the type of test within the range 0.005 25 - 0.05 M.

As reported in a number of papers [4, 5], the ease with which ion exchange takes place and the rapidity with which ion equilibrium is attained for electroconductive polymers immersed in a solution are 30 essentially dependent on the size of the anti-ion introduced at the electrodeposition stage: the larger the ionic radius of the anti-ion, the more readily ion-exchange reactions take place and the more rapidly a state of equilibrium is reached. This is 35 directly linked to the value and rate of change of

- 21 -

- (c) contacting the sensing electrode with a solution comprising competing molecules capable of binding to said immobilized or adsorbed receptors, said competing molecules being conjugated with an enzyme;
- 5 d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and
- 10 e) monitoring the electric potential difference between the sensing electrode and a reference electrode following exposure to an electrolyte comprising the substrate for said enzyme.

In one embodiment of the above-described methods the enzyme conjugated to the secondary receptors or competing molecules is capable of converting a substrate which directly affects the redox composition of the electroconductive polymer coating of the sensing electrode into a product which has no detectable effect on the redox composition of the electroconductive polymer. An example of such an enzyme is horseradish peroxidase.

In an alternative embodiment, the enzyme conjugated to the secondary receptors or competing molecules is capable of converting a substrate which has no detectable effect on the redox composition of the electrochemical polymer coating of the sensing electrode to a product capable of directly or indirectly affecting the redox composition of the electroconductive polymer. One way in which the product of the enzymic reaction may indirectly affect the redox composition of the electroconductive polymer is by causing a change in the pH of the electrolyte (for this embodiment the pH of the electrolyte is not buffered). An example of an

References

- 5 1. Kasparov S.V., Farmakovsky D.A., Kharlamov A.A., Damiryan A.U., Remen V.V. Device for detecting biologically active compounds in biological fluids and a method of manufacturing of the sensing element. Patent of the Russian Federation №2032908.
- 10 2. Kasparov S.V., Farmakovsky D.A. Electrochemical immunoassay. WO 96/02001.
- 15 3. Farmakovsky D.A., Milanovsky E. Yu., Cherkasov V.R., Biryukov Yu. S., Komarov B.V. A method of electrochemical induction of immuno-chemically active macromolecules in test solutions. Patent of the Russian Federation №2107296.
- 20 4. Ge Hailin, Wallace G.G. Ion exchange properties of polypyrrole. Reactive polymers, 18, 133-140, 1992.
- 25 5. Curtin L.S., Komplin G.C., Pietro W.J., Diffusive Anion Exchange in polypyrrole films. J. of Physical Chemistry, 92, 12-13, 1988.
- 30 6. Bobacks J., Gao Zh., Ivaska A., Lewenstam. A., Mechanism of ionic and redox sensitivity of p-type conducting polymers. Part 2. Experimental study of polypyrrole. J. of Electrochemical Chemistry, 368, 33-41, 1994.
- 35

- 87 -

7. Scheller F., Pfeiffer D., Schubert F., Renneberg R., Kirstein D., Use of enzyme amperometric biosensors in the analysis of real subjects. Biosensors: Basic principles and attachments.
5 Moscow.: Mir, 1992.
8. Taniguchi I., Yasukouchi K., Tsuji I., Potential-causing element for immunosensor. EP 10 0 193 154 A2.
9. Hodgson A.J., Spencer M.J., Wallace G.G., Incorporation of proteins into conducting electroactive polymers. Reactive polymers 18, 15 77-85, 1992.
10. John R., Spencer M.J., Wallace G.G., Smyth M.R.. Development of a polypyrrole-based human serum albumin sensor. Antalytica Chimica Acta, 249, 381-385, 1991.
11. Leary J.J., Brigati D.J., Ward D.C., Rapid and sensitive colorimetric method for visualising biotin-labelled DNA probes hybridised to DNA or RNA immobilised on nitrocellulose: bio-blots.
20 Proc. Natl. Academy Science USA, 80, 4045-4049, 25 1983.
12. Rosenstein R., Immuno-agglutination particle suspensions. EP 0 138 297 A1.
30
13. Schasfoort R.B.M., Bergveld P., Bomer J., Kooyman R. P. H., Greve J., Modulation of the ISFET response by an immunological reaction. Sensors and Actuators, 17, 531-535, 1989.

- 88 -

14. Schasfoort R.B.M., Keldermans C.E.J.M., Kooyman R.P.H., Bergveld P., Greve J., Competitive immunological detection of progesterone by means of the ion-step induced response of an ImmunoFET. Sensors and Actuators B1, 368-372, 1990.
- 5
15. Schasfoort R.B.M., Kooyman R.P.H., Bergveld P., Greve J., A New Approach to ImmunoFET Operation. Biosensors and Bioelectronics, 5, 103-124, 1990.
- 10
16. Bergveld P., A critical evaluation of direct protein detection methods. Biosensors and Bioelectronics, 6, 55-72, 1991.
- 15
17. Hager H.J., Latex polymer reagents for diagnostic tests. United States Patent 3,857,931.
- 20
18. Spadaro A.M., Engler Ph. V., Lyophilisation of reagent-coated particles. EP 0 193 208 B1.
19. Harlow E., Lane D., Antibodies: A Laboratory Manual, Cold Spring Harbor, NY, 1988.
- 25
20. Rigby P.J.W., Dieckman M., Rhodes C., Berg R., Labelling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase. J. of Molecular Biology, 113, 237-251, 1977.
- 30
21. Shan S. Wong, Chemistry of Protein Conjugation and Cross-Linking, CRC Press Inc, 1991.

- 89 -

Claims:

1. A sensing electrode for use in methods of electrochemical detection of an analyte, the sensing
5 electrode comprising an electrically conductive electrode coated with a layer of electroconductive polymer with adaptor molecules selected from the group consisting of avidin, streptavidin, anti-FITC antibodies and a molecule capable of specifically
10 binding to at least one class of receptor molecules immobilised therein or adsorbed thereto.

2. A sensing electrode as claimed in claim 1 in which the layer of electroconductive polymer has
15 been doped with anions of large ionic radius.

3. A sensing electrode as claimed in claim 1 or claim 2 wherein the adaptor molecules are avidin or streptavidin and biotinylated receptors capable of
20 binding said analyte are attached thereto via a biotin/avidin or biotin/streptavidin binding interaction.

4. A sensing electrode as claimed in claim 1
25 or claim 2 wherein the adaptor molecules are protein A or protein G and antibodies capable of binding said analyte are attached thereto via a protein A/antibody or protein G/antibody binding interaction.

30 5. A sensing electrode as claimed in claim 1 or claim 2 wherein the adaptor molecules are lectins and receptors capable of binding said analyte are attached thereto via a lectin/carbohydrate binding interaction.

- 90 -

6. A sensing electrode as claimed in claim 1 or claim 2 wherein the adaptor molecules are anti-FITC antibodies and receptors capable of binding said analyte are attached thereto via a FITC/anti-FITC binding interaction.

7. An electrode assembly comprising a sensing electrode as claimed in any one of claims 1 to 6 and a reference electrode.

10 8. A method of producing a sensing electrode for use in methods of electrochemical detection of an analyte, the sensing electrode comprising an electrically conductive electrode coated with an 15 electroconductive polymer with adaptor molecules selected from the group consisting of avidin, streptavidin, anti-FITC antibodies and a molecule capable of specifically binding to at least one class 20 of receptor molecules immobilised therein, the method comprising the steps of:

a) preparing an electrochemical polymerisation solution comprising monomeric units of the electroconductive polymer and adaptor molecules,

25 b) immersing the electrode to be coated in the electrochemical polymerisation solution, and

c) applying a cyclic electric potential 30 between the electrode and the electrochemical polymerisation solution to coat the electrode by electrochemical synthesis of the polymer from the solution, said cyclic electric potential being applied for at least one full cycle.

- 91 -

9. A method of producing a sensing electrode for use in methods of electrochemical detection of an analyte, the sensing electrode comprising an electrically conductive electrode coated with an 5 electroconductive polymer with adaptor molecules selected from the group consisting of avidin, streptavidin, anti-FITC antibodies and a molecule capable of specifically binding to at least one class of receptor molecules adsorbed thereto, the method 10 comprising steps of:

- a) preparing an electrochemical polymerisation solution comprising monomeric units of the electroconductive polymer,
- b) immersing the electrode to be coated in the electrochemical polymerisation solution,
- c) applying a cyclic electric potential between the electrode and the electrochemical polymerisation solution to coat the electrode by electrochemical synthesis of the polymer from the solution, said cyclic electric potential being applied for at least one full cycle; and
- d) contacting the coated electrode with a solution comprising adaptor molecules such that the adaptor molecules are adsorbed onto the electroconductive polymer coating of the electrode.

10. A method as claimed in claim 8 or claim 9 wherein the adaptor molecules are avidin or streptavidin and the method further comprises the step of contacting the sensing electrode with a 35 solution comprising receptor molecules, said receptor

- 92 -

molecules being conjugated with biotin such that said
biotinylated receptors bind to molecules of avidin or
streptavidin immobilised in or adsorbed to the
electroconductive polymer coating of the electrode
5 via a biotin/avidin or biotin/streptavidin binding
interaction.

11. A method as claimed in claim 10 wherein the
receptor molecules are monoclonal antibodies,
10 polyclonal antibodies, antibody fragments, antibody
mimics, chimaeric antibodies viral lysates,
recombinant proteins, synthetic peptides, hormones,
hormone receptors, single stranded nucleic acids, low
molecular weight molecules, chemical compounds
15 conjugated with proteins (haptens), fragments of
bacterial, plant or animal cells, lectins,
glycoproteins or carbohydrates.

12. A method as claimed in claim 8 or claim 9
20 wherein the adaptor molecules are protein A, protein
G or lectins.

13. A method as claimed in any one of claims 8
to 12 wherein the cyclic electric potential has a
25 sawtooth form.

14. A method as claimed in any one of claims 8
to 13 wherein the cyclic electric potential is
applied for at least two cycles.
30

15. A method as claimed in any one of claims 8
to 14 wherein the cyclic electric potential has a
peak value applied to the electrode which is less
than or equal to +2 volts.

- 93 -

16. A method as claimed in any one of claims 8 to 13 in which a salt whose anions have a large ionic radius is added to the electrochemical polymerisation solution.

5

17. A method as claimed in claim 16 wherein the salt is sodium dodecylsulphate or sodium dextran sulphate.

10

18. A method as claimed in any one of claims 8 to 17 wherein the monomeric units of the electroconductive polymer are aniline, thiophene, furan or pyrrole.

15

19. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

20
25

a) providing a sensing electrode having an electroconductive polymer coating, the coating having immobilised therein or adsorbed thereto receptors which are capable of binding the desired analyte to be detected in the sample;

25

b) contacting the sensing electrode with a test solution comprising the sample so that said desired analyte binds to said immobilised or adsorbed receptors;

30
35

c) contacting the sensing electrode with a solution comprising secondary receptors capable of binding to said analyte at a site spatially distinct from the site of binding to the immobilised or adsorbed receptors, said secondary receptors being conjugated with a charge label;

- 94 -

d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and

5

e) monitoring the electric potential difference between the sensing electrode and a reference electrode following a change in the ionic strength of the electrolyte at constant pH.

10

20. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

15

a) providing a sensing electrode having an electroconductive polymer coating, the coating having immobilised therein or adsorbed thereto receptors which are capable of binding to the desired analyte to be detected in the sample;

20

b) contacting the sensing electrode with a test solution comprising the sample so that said analyte binds to said immobilised or adsorbed receptors;

25

c) contacting the sensing electrode with a solution comprising competing molecules capable of binding to said immobilised or adsorbed receptors, said competing molecules being conjugated with a charge label;

30

d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and

35

- 95 -

e) monitoring the electric potential difference between the sensing electrode and a reference electrode following a change in the ionic strength of the electrolyte at constant pH.

5

21. A method as claimed in claim 19 or claim 20 wherein the charge label has the following properties:

10 (i) it carries a net charge at the pH of the electrolyte of part d); and

(ii) the magnitude of this charge changes in response to a change in the ionic strength of the electrolyte at constant pH;

15 22. A method as claimed in claim 21 wherein the charge label is ferrocene, latex microspheres or gold.

20 23. A method as claimed in any one of claims 19 to 22 wherein steps (b) and (c) are performed simultaneously by contacting the sensing electrode with a test solution to which has been added secondary receptors or competing molecules conjugated with a charge label.

25

24. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

30 (a) providing a sensing electrode having an electroconductive polymer coating, the coating having immobilized therein or adsorbed thereto receptors which are capable of binding to the desired analyte to be detected in the sample;

35 (b) contacting the sensing electrode with a

- 96 -

test solution comprising the sample so that the said analyte binds to said immobilized or adsorbed receptors;

5 (c) contacting the sensing electrode with a solution comprising secondary receptors capable of binding to said analyte at a site spatially distinct from the site of binding to immobilized or adsorbed receptors, said secondary receptors being conjugated with an enzyme;

10 d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and

15 e) monitoring the electric potential difference between the sensing electrode and a reference electrode following exposure to an electrolyte comprising the substrate for said enzyme.

25. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

25 (a) providing a sensing electrode having an electroconductive polymer coating, the coating having immobilized therein or adsorbed thereto receptors which are capable of binding to the desired analyte to be detected in the sample;

30 (b) contacting the sensing electrode with a test solution comprising the sample so that the said desired analyte binds to said immobilized or adsorbed receptors;

- 97 -

5 (c) contacting the sensing electrode with a solution comprising competing molecules capable of binding to said immobilized or adsorbed receptors, said competing molecules being conjugated with an enzyme;

10 (d) monitoring the electric potential difference between the treated sensing electrode and a reference electrode when both are immersed in an electrolyte; and

15 (e) monitoring the electric potential difference between the sensing electrode and a reference electrode following exposure to an electrolyte comprising the substrate for said enzyme.

20 26. A method as claimed in claim 24 or claim 25 wherein the enzyme is capable of converting a substrate which directly affects the redox composition of the electroconductive polymer coating of the sensing electrode to a product which has no detectable effect on the redox composition of the said electroconductive polymer coating.

25 27. A method as claimed in claim 26 wherein the enzyme is a peroxidase.

30 28. A method as claimed in claim 24 or claim 25 wherein the enzyme is capable of converting a substrate which has no detectable effect on the redox composition of the electroconductive polymer coating of the sensing electrode to a product capable of directly or indirectly affecting the redox composition of the said electroconductive polymer coating.

35

- 98 -

29. A method as claimed in claim 28 wherein the product capable of indirectly affecting the redox composition of the electroconductive polymer membrane causes a change in the pH of the electrolyte of part
5 (e).

30. A method as claimed in claim 29 wherein the enzyme is a urease.

10 31. A method as claimed in claim 24 or claim 25 wherein the enzyme is capable of converting a substrate which has no detectable effect on the redox composition of the electroconductive polymer coating of the sensing electrode to a product which is a
15 substrate for a second enzyme, the action of the second enzyme generating a second product which directly or indirectly affects the redox composition of the electroconductive polymer coating of the sensing electrode.

20 32. A method as claimed in any one of claims 24 to 31 wherein steps (b) and (c) are performed simultaneously by contacting the sensing electrode with a test solution to which has been added
25 secondary receptors or competing molecules conjugated with an enzyme label.

30 33. A method as claimed in any one of claims 19 to 32 wherein the receptors capable of binding to the analyte to be detected are biotinylated and are attached to avidin or streptavidin immobilised in or adsorbed to the electroconductive polymer coating of the sensing electrode via a biotin/avidin or biotin/streptavidin binding interaction.

- 99 -

34. A method as claimed in any one of claims 19
to 32 wherein the receptors capable of binding to the
analyte to be detected are antibodies and are
attached to protein A or protein G immobilized in or
5 adsorbed to the electroconductive polymer coating of
the sensing electrode via a protein A/antibody or
protein G/antibody binding interaction.

10 35. A method as claimed in any one of claims 19
to 32 wherein the receptors capable of binding to the
analyte to be detected contain a sugar moiety and are
attached to lectins immobilized in or adsorbed to the
electroconductive polymer coating of the sensing
electrode via a lectin/sugar binding interaction.

15 36. A method as claimed in any one of claims 19
to 32 wherein the receptors capable of binding to the
analyte to be detected are labelled with FITC and are
attached to anti-FITC antibodies immobilized in or
20 adsorbed to the electroconductive polymer coating of
the sensing electrode via a FITC/anti-FITC binding
interaction.

25 37. A method as claimed in any one of claims 19
to 36 wherein the sensing electrode is produced
according to the method of any one of claims 8 to 18.

30 38. A method as claimed in claim 33 wherein
steps (b) and (c) are performed simultaneously with a
step of contacting the sensing electrode with
biotinylated receptors by contacting the sensing
electrode with a test solution to which has been
added biotinylated receptors and secondary receptors
or competing molecules conjugated with a charge label
35 or an enzyme.

- 100 -

39. A method as claimed in claim 19 wherein the secondary receptors are polyclonal antibodies or monoclonal antibodies.

5 40. A method as claimed in any one of claims 19 to 39 in which biological fluids such as whole blood, serum, lymph, urine, saliva, cerebrospinal fluid and semen are used as the test solution.

10 41. A method of electrochemical detection of an analyte in a sample, which method comprises the steps of:

15 (a) providing a sensing electrode comprising an electrically conductive electrode coated with a layer of electroconductive polymer with molecules of avidin or streptavidin immobilized therein or adsorbed thereto, said avidin or streptavidin molecules being attached to receptor molecule capable of binding the analyte to be detected attached via a 20 biotin/avidin or biotin/streptavidin binding interaction;

25 (b) contacting the sensing electrode with a test solution comprising the sample so that said desired analyte binds to said immobilized or adsorbed receptor molecules;

30 (c) monitoring the potential of the sensing electrode relative to a reference electrode when both are immersed in an electrolyte; and

35 (d) monitoring the potential difference of the sensing electrode relative to the reference electrode following a change in the ionic strength or composition of the electrolyte at constant pH.

- 101 -

42. A method as claimed in claim 41 wherein the analyte to be detected is a nucleic acid and the receptor molecules are oligonucleotides.